REMARKS

Status of the Prosecution:

Claims 16-26, 30 and 31 are pending. Claims 30 and 31 are withdrawn as drawn to nonelected inventions. Claims 17, 18 and 26 have been canceled. Claims 16, and 18-25 remain pending and under examination. The drawings have been accepted and the Applicants acknowledge this with thanks. In addition, the Applicants wish to express appreciation for the returned, initialed Information Disclosure Sheets.

Claims 16 and19 have been amended herein.

Claim 16 is Directed Solely to Elected Subject Matter

Claim 16 stands objected to as reciting nonelected enzymes. Applicants have amended claim 16, which no longer recites nonelected enzymes. Accordingly, the grounds of the objection are moot. Reconsideration is respectfully requested.

The Claimed Invention is Adequately Described Within the Meaning of 35 U.S.C. § 112, first paragraph.

Claims 16-18 and 20-25 stand rejected as allegedly failing to comply with the written description requirement of 35 U.S.C. § 112, first paragraph. Applicants respectfully traverse this rejection. The Office Action alleges that the Applicants did not have possession of the invention, particularly stating the claims recite any p-hydroxybenzaldehyde synthase from any source, and functional variants thereof.

Claim 16 and claims dependent thereon are presently directed to methods of improving vanillin production involving genetically engineering *V. planifolia* to overproduce p- hydroxybenzaldehyde synthase enzymes wherein the enzyme has the sequence of SEQ ID NO:2, and , and further to methods wherein the enzyme is encoded by SEQ ID NO:1. The claims no longer recite functional derivatives of such enzymes.

The enzymes as claimed in the invention are clearly described in the specification and the working examples provide examples of the enzymes – the written description of 35 U.S.C. § 112, first paragraph requires nothing more. Accordingly, the claims meet the

requirements of 35 U.S.C. § 112, first paragraph for the written description and the Applicants respectfully request reconsideration and withdrawal of the rejection.

The Claimed Invention is Fully Enabled Under 35 U.S.C. § 112, first paragraph.

Claims 16-25 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly containing subject matter not described in such a way as to enable the skilled artisan to make and use the invention. In particular, the Office Action acknowledges that the specification teaches genetically engineering bacteria, yeast, *Arabidopsis* and higher plants with the claimed sequences (e.g. the nucleic acid of SEQ ID NO:1). The Office Action apparently alleges that the skilled artisan would not be able to assess the effect of expressing the encoded polypeptide on the production of vanillin in any cellular or organismal system. In addition the Office Action alleges that the specification does not enable the use of functional variants of the p-hydroxybenzaldehyde synthase enzyme.

Applicants respectfully traverse the rejection. With respect to the enablement of functional variants, Applicants respectfully note that the claims as amended do not recite any functional variants as discussed above under written description. To the extent the rejection is based on functional variants, reconsideration is respectfully requested.

As pertains to the enablement of genetically tranforming *V. planifolia*, the Office Action acknowledges that the specification is enabling for genetically engineering *V. planifolia* to overproduce the chain shortening enzyme by transformation with SEQ ID NO:1, it alleges however that the specification provides no guidance for other methods of genetic engineering, such as breeding, for example. The skilled artisan clearly understands that the techniques of genetic engineering and manipulation are not sequence specific and can be broadly applied to any sequence, including the novel sequences disclosed herein. Applicants respectfully assert that the sequences provided can be used in a variety of genetic engineering methods and that Applicants need not teach what is well-known (the methods) which can be applied with the sequences to produce a plant or plant cells expressing the sequence. Given the sequences provided in the specification, the skilled artisan can apply any of the well-established techniques for genetic engineering (see for example page 25, lines 15- 30 reciting and providing references for a wide variety of methods of generating transgenic plants) and can powerfully select for the desired transgenic plant or cell. This would not require

inventive effort or undue experimentation but rather would be only routine. Since Applicants specification must only enable one to make and use the invention and need not eliminate routine efforts on the part of the skilled artisan, the requirements 35 U.S.C. § 112, first paragraph are satisfied. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph with respect to the use of genetic engineering methods for introducing the nucleic acid of SEQ ID NO:1, or expressing the protein of SEQ ID NO:2.

With respect to the enablement of determining the effect of expressing the encoded polypeptide on the production of vanillin in a cellular system, Applicants respectfully assert that the guidance in the specification is adequate to enable the skilled artisan to practice the invention with undue experimentation. The Office Action alleges that it would be unpredictable as to whether the overproduction of an enzyme associated with chain shortening of p-coumaric acid to p-hydroxybenzaldehyde would improve production of vanillin in *V. planifolia*, as the chain shortening is but one of several steps required for vanillin biosynthesis.

The Office Action cites Havkin-Frenkel *et al.* (Food technology 1997 51(11) pages 56-58, 61) for the proposition that roughly 80% of coumaric acid applied to cultured *V. planifolia* cells was recovered as p-hydroxybenzyl alcohol (HBA) whereas feeding of proaldehyde led to the accumulation of vanillin. The Office Action also cites Havkin-Frenkel for the proposition that the hydroxylation of HBA to pro-aldehyde is a limiting step in vanillin biosynthesis. The Office Action further cites the instant specification for teaching that more than one enzyme is required for vanillin biosynthesis (Figure 1) and that hydroxylation of HBA to proaldehyde is the rate limiting step in vanillin biosynthesis (page 15, lines 2-6, page 16 lines 6-9), and further that conversion of 4-coumaric acid to 4-hydroxybenzalehyde is not considered to be rate limiting in cultured cells (page 20, lines 6-11). In particular, Applicants respectfully note that the cited portions of the specification from page 15 and 16 relate to "Improving vanillin production in tissue culture by manipulation of culture conditions" and do not necessarily equate to manipulation of enzymes of the vanillin biosynthetic pathway. Further, Applicants note that the text on page 20 states that, with respect to the conversion of 4-coumaric acid to 4-hydroxybenzaldehyde, "this reaction may play a more important rate-controlling function in intact vanilla beans."

Further, the disclosure at page 32, lines 13 - 17 indicates that "it is clear that at least one chain shortening enzyme is involved in the conversion from CA to BA, and that this step does not appear to be rate-limiting in cultured cells. However, some evidence indicates that it is the rate-limiting step in intact vanilla beans."

Further, this whole line of reasoning is apparently predicated on the idea that only overproducing a, or *the*, rate-limiting step enzyme can be helpful in improving production of the end-product of a biosynthetic pathway. Rather, Applicants respectfully assert that the skilled artisan could readily appreciate that improving vanillin production could result, for example, by driving a greater percentage of the p-coumaric acid to p-hydroxybenzaldehyde, which in turn could drive the next reaction step to the HBA side of the scheme, and further downstream to vanillin. Furthermore, overproduction of the chain shortening enzyme could readily be envisioned to for example offset the affect of VAD as indicated on page 55, which can impact the amount of 4-hydroxybenzaldehyde, by dehydrogenation. Overproduction of the enzyme which produces 4-hydroxybenzaldehyde (from 4-coumaric acid) would be help maintain concentrations and keep the main pathway supplied, thereby improving vanillin production. Additionally, the data from kinetic experiments (see e.g. Example 12, page 68, lines 18-27 suggest that positive cooperativity is feature of the chain shortening enzyme. Such a feature is consistent with a multimer, and increasing the production of subunits is likely to enable the multimeric and positively cooperative units to form and function.

Other disclosure also enables the skilled artisan to make and use the invention for improving vanillin production with an expectation of success. For example, on page 69 it is stated that "non-oxidative chain shortening appears to be the major route to vanillin precursors in *V. planifolia* cell cultures." Thus, the skilled artisan would appreciate that by increasing production of the chain shortening enzyme, the vanillin precursors could be increased, thus improving the production of vanillin. Again at worse case, no undue experimentation is required. The skilled artisan could resort to routine measurement of vanillin by art-known methods to determine whether the genetic engineering of the *V. planifolia* had been successful in improving vanillin production.

Accordingly, the Applicants respectfully request reconsideration of the rejection under 35 U.S.C. § 112, first paragraph for lack of enablement. The skilled artisan would be able to make and use the full range of the claimed invention to improve vanillin production

based on the specification and the working examples and applying only routine efforts. Withdrawal of the rejection is respectfully requested.

Conclusions:

This amendment is believed to be fully responsive to all outstanding issues and Applicants respectfully assert that all claims are now in condition for allowance. An early and favorable notice to that end is earnestly solicited. The Examiner is invited to contact the Applicants undersigned representative by telephone at 215-557-5986 to resolve any outstanding matters prior to allowance.

Respectfully submitted,

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